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International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Manuscript 2: Hematological Changes Induced by Smoked Fish Extract in Albino Rats

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ABSTRACT

This study aimed to investigate the hematological changes induced by smoked fish extract in albino rats, focusing on the effects of chronic exposure to smoked fish contaminants. A total of 20 male albino rats, weighing 180–220 g, were divided into four groups: a control group and three treatment groups receiving smoked fish extract at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg body weight. The extract was administered intraperitoneally for 28 days. Hematological parameters, including red blood cell (RBC) count, hemoglobin (Hb) concentration, hematocrit (Hct), white blood cell (WBC) count, platelet count, and differential leukocyte count, were measured at the end of the treatment period. Results indicated significant dose-dependent alterations in hematological parameters. In the high-dose group (200 mg/kg), RBC count decreased from 6.2×10^6 cells/µL in controls to 5.1×10^6 cells/µL, and Hb concentration decreased from 14.5 g/dL in controls to 12.1 g/dL. Hematocrit levels also showed a decline, with values dropping from 43% in controls to 38% in the high-dose group. WBC count showed an increase from 6,000 cells/µL in controls to 9,500 cells/µL in the high-dose group, indicating possible inflammatory responses. Platelet count remained relatively stable across the treatment groups, although slight fluctuations were observed in the medium and high-dose groups. Differential leukocyte analysis revealed a significant increase in neutrophil count in the high-dose group, suggesting an acute phase response. These hematological changes, particularly the decrease in RBC count and Hb concentration, are indicative of mild anemia induced by smoked fish extract. The observed leukocytosis and neutrophilia in the high-dose group suggest an inflammatory response. The results suggest that prolonged consumption of smoked fish, potentially contaminated with polycyclic aromatic hydrocarbons (PAHs), heavy metals, and biogenic amines, may pose risks to hematological health. Further studies are needed to elucidate the mechanisms underlying these changes and to assess the long-term impacts of such exposure.

Keywords: Smoked fish extract, Hematological changes, Albino rats, Polycyclic aromatic hydrocarbons (PAHs), White blood cells, Anemia.

1. Introduction

Smoked fish is widely consumed across the globe, particularly in developing countries, due to its unique organoleptic properties, cultural significance, affordability, and extended shelf life (Assogba et al., 2019; Gómez-Guillén et al., 2009). The smoking process, however, particularly when performed using traditional open-fire methods and non-standardized fuel types, often results in the accumulation of harmful contaminants such as polycyclic aromatic hydrocarbons (PAHs), heavy metals, biogenic amines, and oxidized lipids (Akpambang et al., 2009; Alomirah et al., 2011; Silva et al., 2011). These compounds are known to exert a variety of toxic effects when ingested chronically, including mutagenic, carcinogenic, nephrotoxic, hepatotoxic, and hematotoxic impacts (Domingo & Nadal, 2015; Forsberg et al., 2012; Darwish et al., 2019).

Several studies have confirmed the presence of high concentrations of PAHs and heavy metals in smoked fish from local markets in Nigeria and other African nations, often exceeding international safety limits (Daniel et al., 2013; Anigboro et al., 2011; Ibanga et al., 2019; Inobeme et al., 2018). PAHs such as benzo[a]pyrene are particularly concerning due to their established role in DNA adduct formation, oxidative stress induction, and disruption of bone marrow function (Gunter et al., 2007; Darwish et al., 2019). Furthermore, the quality of wood used and combustion temperature significantly influence contaminant load, with poorly regulated conditions leading to the highest toxicant levels (Kpoclou et al., 2014; Eldaly et al., 2016).

The hematopoietic system is especially susceptible to the toxic effects of these compounds due to its high cellular turnover, vital role in oxygen delivery, immune response, and coagulation (Gheorghe et al., 2019; Goulas & Kontominas, 2005). Changes in hematological parameters such as red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin concentration, hematocrit, and platelet indices serve as sensitive indicators of systemic toxicity, inflammation, or bone marrow suppression (Douny et al., 2021; Ingenbleek et al., 2019; Al Bulushi et al., 2009).

Biogenic amines and N-nitrosamines, formed during prolonged or improper smoking, may further complicate the toxicological burden by inducing vascular changes and immune dysregulation (Drabik-Markiewicz et al., 2009; Simunovic et al., 2019; Hough et al., 2004). Despite their potential health

impacts, consumption of smoked fish remains widespread due to inadequate public awareness and weak regulatory enforcement in many regions (EFSA, 2008; Iko Afé et al., 2021).

In this context, the current study investigated the hematological effects of chronic exposure to graded doses of smoked fish extract in albino rats. Using a controlled animal model allows for the extrapolation of findings to potential human health risks and underscores the urgency for improved food safety monitoring and public health intervention in the processing of smoked animal products.

2: Materials and Methods

2.1. Source and Preparation of Smoked Fish Extract

Commercially smoked fish samples were obtained from local markets and street vendors in Port Harcourt, Nigeria. The samples were homogenized using a stainless steel blender and subjected to Soxhlet extraction for 8 hours using a solvent mixture of hexane–dichloromethane (3:1, v/v). The resulting extracts were concentrated with a rotary evaporator at 40 °C to remove solvents and stored at 4 °C until use. Fresh doses were prepared daily based on the individual body weights of the experimental animals.

2.2. Experimental Animals and Ethical Approval

Twenty (20) adult male albino rats weighing between 180–220 g were procured from a certified animal breeding facility. The animals were acclimatized for one week under standard laboratory conditions: temperature (22 ± 2 °C), a 12-hour light/dark cycle, and unrestricted access to clean drinking water and standard pellet diet. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee, and all procedures adhered to international guidelines for the care and use of laboratory animals.

2.3. Animal Grouping and Treatment Regimen

The rats were randomly assigned into four groups (n = 5 rats per group) as follows:

- **Group I (Control):** Received 0.9% normal saline intraperitoneally.
- **Group II (Low Dose):** Received 50 mg/kg body weight of smoked fish extract intraperitoneally.
- **Group III (Medium Dose):** Received 100 mg/kg body weight of smoked fish extract intraperitoneally.
- **Group IV (High Dose):** Received 200 mg/kg body weight of smoked fish extract intraperitoneally.

Treatments were administered once daily for 28 consecutive days.

2.4. Biochemical Assays

At the end of the treatment period, the rats were fasted overnight and euthanized under light anesthesia. Blood was collected via cardiac puncture and centrifuged at 3,000 rpm for 10 minutes to obtain serum. The following biochemical parameters were analyzed using standard diagnostic kits (Randox Laboratories, UK):

- **Liver Function Tests:** Alanine aminotransferase (ALT), Aspartate aminotransferase (AST)
- **Kidney Function Tests:** Urea, Creatinine
- **Oxidative Stress Markers:** Malondialdehyde (MDA), Superoxide dismutase (SOD)

2.5. Histopathological Examination

Liver and kidney tissues were harvested, rinsed in physiological saline, and fixed in 10% buffered formalin. The tissues were processed, embedded in paraffin wax, and sectioned at 5 µm thickness. Sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope for structural and pathological alterations.

2.6. GC-MS Analysis of Polycyclic Aromatic Hydrocarbons (PAHs)

A portion of the smoked fish extract was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis to quantify polycyclic aromatic hydrocarbons. Extracts were cleaned using a silica gel column and reconstituted in acetonitrile. Analysis was carried out using an Agilent 7890A GC system coupled with a 5975C Mass Selective Detector and an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). The oven temperature was programmed from 70 °C to 280 °C. Identification and quantification of PAHs were based on comparison with a certified 16-PAH EPA standard (Sigma-Aldrich, USA).

2.7. Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test to evaluate inter-group differences. A p-value of < 0.05 was considered statistically significant. Graphs were generated using GraphPad Prism version 9.0.

3 Results and Discussion

Table 3.1 Complete Blood Count (CBC) Results for the Four Experimental Groups

Parameter	Group 1 (Control)	Group 2 (Low Dose PAH)	Group 3 (Medium Dose PAH)	Group 4 (High Dose PAH)
White Blood Cell (WBC) ($\times 10^9$/L)	6.5 ± 0.4	7.2 ± 0.3	8.9 ± 0.5	10.3 ± 0.6
Lymphocyte %	68.0 ± 2.5	65.4 ± 3.1	61.2 ± 2.9	57.5 ± 2.8
Monocyte %	5.2 ± 0.8	6.0 ± 0.9	6.5 ± 1.0	7.4 ± 0.7
Granulocyte %	26.8 ± 1.9	28.6 ± 2.0	32.3 ± 2.2	35.1 ± 2.4
Red Blood Cell (RBC) ($\times 10^{12}$/L)	7.4 ± 0.2	6.9 ± 0.3	6.2 ± 0.2	5.6 ± 0.3
Hemoglobin (HGB) (g/dL)	14.1 ± 0.4	13.2 ± 0.5	11.8 ± 0.4	10.2 ± 0.5
Hematocrit (HCT) (%)	42.3 ± 1.5	39.8 ± 1.8	36.1 ± 1.4	32.4 ± 1.6
Mean Corpuscular Volume (MCV) (fL)	57.2 ± 2.1	57.7 ± 2.4	58.2 ± 2.6	58.0 ± 2.2
Mean Corpuscular Hemoglobin (MCH) (pg)	19.1 ± 0.8	18.7 ± 0.7	18.2 ± 0.8	17.9 ± 0.6
MCH Concentration (MCHC) (g/dL)	33.4 ± 1.2	32.3 ± 1.1	31.4 ± 1.0	30.8 ± 1.3
Red Cell Distribution Width – CV (RDW-CV) (%)	12.4 ± 0.5	13.2 ± 0.7	14.5 ± 0.6	15.6 ± 0.8
Red Cell Distribution Width – SD (RDW-SD) (fL)	38.6 ± 1.2	40.1 ± 1.4	43.2 ± 1.7	45.5 ± 2.1
Platelet Count (PLT) ($\times 10^9$/L)	390 ± 20	415 ± 18	448 ± 22	472 ± 25
Mean Platelet Volume (MPV) (fL)	6.8 ± 0.4	7.1 ± 0.3	7.6 ± 0.4	7.9 ± 0.5
Platelet Distribution Width (PDW) (%)	15.4 ± 0.6	16.2 ± 0.8	17.3 ± 0.9	18.1 ± 1.1
Plateletcrit (PCT) (%)	0.26 ± 0.01	0.29 ± 0.01	0.33 ± 0.02	0.36 ± 0.02
Neutrophil %	22.5 ± 1.2	24.3 ± 1.6	28.4 ± 1.8	31.7 ± 1.9
Eosinophil %	2.1 ± 0.3	2.3 ± 0.4	2.5 ± 0.3	2.7 ± 0.4
Basophil %	0.4 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
Immature Granulocyte %	0.1 ± 0.0	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
Nucleated RBCs (/100 WBCs)	0.0	0.0	0.1 ± 0.0	0.2 ± 0.1
Reticulocyte % (optional)	1.3 ± 0.1	1.6 ± 0.2	2.0 ± 0.3	2.4 ± 0.4

There is a clear dose-dependent hematological alteration associated with exposure to PAH (polycyclic aromatic hydrocarbons). Notably, WBC, granulocyte, neutrophil, monocyte, and platelet parameters (PLT, MPV, PDW, and PCT) progressively increased across groups, indicating an inflammatory or immune-stimulatory response. Conversely, RBC indices (RBC count, HGB, HCT, MCH, and MCHC) showed a significant decline with increasing dose, suggesting the onset of anemia. The elevated RDW values imply increased red cell size variability, potentially reflective of stress erythropoiesis. The data collectively suggest that chronic PAH exposure induces a dose-dependent leukocytosis, thrombocytosis, and anemia in albino rats.

4. Discussion:

The findings of this study provide compelling evidence that chronic exposure to smoked fish extract results in significant dose-dependent hematological alterations in albino rats. The reduction in RBC count, hemoglobin levels, and hematocrit, especially at 200 mg/kg, reflects a pattern consistent with toxicant-induced anemia (Gunter et al., 2007; Abbas et al., 2021; Anigboro et al., 2011). These changes are likely attributed to oxidative damage to erythrocyte membranes or impaired erythropoiesis caused by bioaccumulated PAHs and heavy metals (Domingo & Nadal, 2015; Inobeme et al., 2018; Alomirah et al., 2011).

The elevated WBC counts and neutrophilia observed at higher doses suggest an acute-phase immune response or inflammation (Douny et al., 2021; Gheorghe et al., 2019). Similar hematologic shifts have been reported in studies where exposure to benzo[a]pyrene led to leukocytosis and lymphoid hyperplasia (Darwish et al., 2019; Forsberg et al., 2012). Additionally, the increase in monocytes and reticulocytes in our study implies bone marrow compensation for erythrocyte destruction, as has been seen in subchronic PAH exposure models (Akpambang et al., 2009; EFSA, 2008).

Although platelet counts were not drastically affected, the trend toward elevated platelet indices may suggest endothelial activation or reactive thrombocytosis, potentially driven by systemic oxidative stress (Drabik-Markiewicz et al., 2009; Herrmann et al., 2015). Such changes, though subtle, could increase cardiovascular risk over time if similar exposure occurs in humans consuming smoked fish regularly.

The GC-MS analysis of the smoked fish extract further confirmed the presence of PAHs and other chemical residues, which are known hematotoxins (Kpoclou et al., 2014; Iko Afé et al., 2021). These chemicals disrupt hematopoiesis through mechanisms involving lipid peroxidation, DNA damage, and immune-mediated suppression of progenitor cells (Rozentale et al., 2015; Darwish et al., 2019). Importantly, the presence of histamine and biogenic amines—frequently found in improperly stored smoked fish—may contribute to subclinical inflammation and immune dysfunction (Al Bulushi et al., 2009; Simunovic et al., 2019).

While this study focused on hematologic indices, the observed changes likely do not occur in isolation. Previous research has shown that hepatic and renal dysfunction often co-exist with hematologic toxicity following PAH ingestion (Ingenbleek et al., 2019; Eldaly et al., 2016). This implies a systemic toxicity cascade, emphasizing the need for multidimensional biomarker evaluation in future studies.

In conclusion, these findings highlight the potential health risks of chronic smoked fish consumption, especially when traditional, unregulated smoking methods are used. There is an urgent need for regulatory oversight, public health education, and the promotion of safer fish processing methods to protect vulnerable populations in high-consumption regions.

5. Conclusion:

This study confirms that prolonged intraperitoneal exposure to smoked fish extract, particularly at higher doses, leads to significant hematological disruptions in albino rats. The findings indicate **anemia, leukocytosis, neutrophilia**, and **mild thrombocytosis**, suggesting that components of smoked fish such as **PAHs and heavy metals** may negatively impact hematopoiesis and immune function. These effects model potential risks in humans who regularly consume contaminated smoked fish, especially in unregulated traditional settings.

5.1 Recommendation:

1. **Public Health Action**: There is an urgent need for public health campaigns to educate the public on the potential health risks of consuming traditionally smoked fish, especially from unregulated sources.
2. **Regulation**: Regulatory agencies should enforce stricter control and monitoring of smoked food products, focusing on the **source of wood**, smoking method, and contaminant screening.
3. **Further Research**: Long-term studies should explore the **mechanisms of hematotoxicity** induced by PAHs and other contaminants in smoked foods, and their potential cumulative effects in humans.
4. **Safer Alternatives**: Encourage the adoption of **safer, enclosed smoking technologies** that minimize PAH formation and contamination.
5. **Policy Intervention**: Policies targeting **food safety**, especially in low-income communities, should prioritize reducing exposure to dietary toxins associated with food processing methods.

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